chapter 1

Microbiology of ready-to-eat foods

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1.1 Introduction

Ready-to-eat (RTE) foods are those food products that have gone through some kind of processing and can be consumed without undergoing any further bactericidal treatment such as thorough heating. These foods do not require further preparation prior to consumption, except washing, thawing, or moderate reheating (Farber and Harwig, 1996). As the name implies, RTE foods can be readily consumed without further preparation or processing and thus are extremely convenient for present-day, busy

consumers. Surveys of consumer purchase behaviors show an increasing trend in the consumption of RTE foods (Anonymous, 2001, 2003). Hence, the demand for these products from the food industry is huge. Ready-to-eat sandwiches account for 32% of sales from vending machines and include a large share of a multibillion-dollar annual business in the United States (Anonymous, 2001). Supermarkets and convenience stores carry a large selection and variety of RTE food products.

Some examples of RTE foods include the following: meats (deli meats, sausages, hot dogs, corned beef), poultry (buffalo chicken, chicken wraps, deli cuts, chicken salad), dairy products (cheeses, yogurt, sour cream, pasteurized milk), fruits and vegetables (salads and leafy greens, salsa, juices, guacamole), and fish and seafood (cold smoked salmon, sushi, seafood salad, fish soup). Some frozen foods can be included among RTE foods, for example, ice cream, frozen yogurt, frozen fruits, and smoothies. Other non-refrigerated RTE foods include products such as breads, nuts, peanut butter, chocolate, and some snack items.

Due to the increasing demand for and consumption of RTE foods, and owing to the fact that these are not further processed, the microbiological risks to the consumer from these products have also increased. The preparation of RTE sandwiches, salads, and meats involves human handling (cutting or slicing), which can easily contribute to cross-contamination. Ready-to-eat food products have been involved in numerous foodborne illness outbreaks, especially due to cross-contamination of these products during processing and packaging in the food processing plant or handling in consumer households. For example, the *Salmonella enterica* outbreak in late 2008 and early 2009 involving peanut butter was due to heavy rainfall through a leaky roof and a faulty sprinkler system in the processing plant, which provided moisture for the growth of dormant salmonellae that were likely present in raw peanuts or peanut dust.

The most common foodborne pathogenic bacteria found in refrigerated RTE foods include *Listeria monocytogenes, Salmonella enterica, Escherichia coli* O157:H7, and *Clostridium perfringens. Listeria monocytogenes* has caused a number of outbreaks and recalls in dairy and RTE meat products. *Salmonella enterica* and *E. coli* O157:H7 have caused outbreaks due to contamination in fresh produce and salads. *Salmonella enterica* has also caused outbreaks and recalls due to cross-contamination in some non-refrigerated RTE foods such as chocolate, almonds, pistachios, peanut butter–containing products, and infant formula. Improper cooling of prepared RTE foods in large volumes is believed to be the most common cause of *C. perfringens* outbreaks. In this chapter, the microbiology of RTE foods is discussed, with emphasis on four main pathogens: *L. monocytogenes, S. enterica, E. coli* O157:H7, and *C. perfringens.*

1.2 Listeria monocytogenes

Listeria monocytogenes is a facultative, intracellular, Gram-positive bacterium and is ubiquitous in the environment. It is a non-spore-forming and psychrotrophic bacterium that produces flat, dimpled colonies with a black halo on modified Oxford formulation (MOX) agar. *Listeria monocytogenes* is a hardy pathogen and can resist various stresses in the environment. It is well known for forming biofilms, and once *L. monocytogenes* forms biofilms in food processing environments, it is very difficult to eradicate the pathogen from such environments. Hence, *Listeria* biofilms can be a threat to food processors. *Listeria monocytogenes* causes listeriosis in humans, which is accompanied by mild flu-like symptoms such as headache, chills, and fever, along with gastrointestinal symptoms like nausea, vomiting, and diarrhea. Immunocompromised people, infants, pregnant women, and elderly people are more at risk for contracting the disease. Meningitis, abortion, and prenatal septicemia are some of the primary manifestations of listeriosis (FDA, 2001a), which in serious cases can be fatal. In serious cases, especially if untreated, mortality may exceed 25% in predisposed groups (Farber and Harwig, 1996). In healthy individuals, *L. monocytogenes* can cause febrile gastroenteritis characterized by fever, headache, muscle and joint pain, and diarrhea (Sim et al., 2002). In febrile gastroenteritis, usually the organisms are found in high numbers in implicated foods and, when ingested, can cause illness in healthy individuals. Due to differences in virulence of strains and host factors such as age, health, and exposure to certain foods, the minimum infectious dose for *L. monocytogenes* is not known (NACMCF, 1991).

1.2.1 *Outbreaks of* L. monocytogenes *in RTE foods*

Several foodborne outbreaks since the 1980s have been attributed to *L. monocytogenes* in the United States and many other countries (CDC, 1999). In the United States, listeriosis has been estimated to cause 2,500 cases of foodborne illness annually, resulting in deaths of 500 people and about $200 million in monetary losses (CDC, 2002; FDA, 2001a; Mead et al., 1999). In the 1980s outbreaks of *L. monocytogenes* associated with cheese and milk were of concern to the dairy industry. Soft cheeses made from raw and pasteurized milk such as Mexican-style cheese (Bula et al., 1995; Linnan et al., 1988) have been involved in listeriosis outbreaks. The Mexican-style cheese outbreak of listeriosis that occurred in 1985 resulted in 142 cases, including 48 deaths (Linnan et al., 1988). In 1982, coleslaw made from cabbages contaminated with sheep manure caused an outbreak of listeriosis in 41 people (Schlech et al., 1983).

In 1997, in Italy, a cold corn and tuna salad caused an outbreak of febrile gastroenteritis due to *L. monocytogenes* contamination (Aureli et al., 2000). Other food products that have been involved as vehicles of non-invasive febrile gastroenteritis due to *L. monocytogenes* contamination include smoked mussels (Misrachi et al., 1991), shrimp (Riedo et al., 1994), rice salad (Salamina et al., 1996), chocolate milk (Dalton et al., 1997), cold smoked rainbow trout (Miettenen et al., 1999), and imitation crab meat (Farber et al., 2000). In these outbreaks, a large number of organisms were suspected to have been consumed by patients. For example, in the chocolate milk outbreak, the median numbers consumed were suspected to be as high as 10^{11} (Dalton et al., 1997). The issue with some of the RTE foods is that they may get cross-contaminated during handling and may initially contain small numbers of organisms. However, because *L. monocytogenes* is psychrotrophic, a high number of organisms may appear during storage, even at refrigeration temperatures. The shelf life of some RTE foods, especially RTE meats, is as long as 2 to 3 weeks, which gives sufficient time for the organism to multiply and probably cause illness, even in immunocompetent individuals.

There have been many outbreaks of *L. monocytogenes* in RTE meat products. In 1992, there was an outbreak of listeriosis in France due to contaminated jellied pork tongue, in which there were 279 reported cases, 63 deaths, and 22 miscarriages or abortions (Jacquet et al., 1995). The listeriosis outbreak in 1998–1999 in the United States resulted in more than 100 cases of illness, including 21 deaths, in over 14 states and was caused by consumption of post-processing contaminated cured meat products. *Listeria monocytogenes* can contaminate cured meat products and grow to high numbers during refrigerated storage (Beumer et al., 1996; Buncic et al., 1991). This is because the bacterium is able to multiply at temperatures as low as 2°C with curing salts and under low oxygen tension (Lou and Yousef, 1999). Another multistate outbreak of listeriosis in 2000 involved delicatessen sliced processed turkey meat that resulted in 30 cases of illnesses, 4 deaths, and 3 miscarriages or stillbirths (CDC, 2000; Olsen et al., 2005). This outbreak was traced to a single processing plant, and the industry recalled 16 million pounds of processed turkey meat. In New Zealand, in 2000, a series of incidents (30 cases) of non-invasive febrile gastroenteritis caused by *L. monocytogenes* were traced to RTE meats, including luncheon ham and corned beef, and high levels of organisms were isolated from patient fecal samples and from implicated meats (Sim et al., 2002). In 2001, another outbreak of febrile gastroenteritis associated with delicatessen meat (precooked sliced turkey) contaminated with *L. monocytogenes* occurred in the southwestern United States (Frye et al., 2002).

A multistate outbreak of *L. monocytogenes* infections in the northeastern United States was caused by the consumption of sliced turkey

deli meat in 2002 (Gottlieb et al., 2006). This outbreak resulted in 54 confirmed cases, 8 deaths, and 3 stillbirths. The outbreak strain was found in one processing plant and the industry recalled more than 30 million pounds of turkey deli meat. This outbreak prompted the Food Safety and Inspection Service (FSIS), an agency of the U.S. Department of Agriculture (USDA), to issue new regulations, including testing and control programs for *L. monocytogenes* in RTE meat and poultry processing plants.

The previously mentioned outbreaks suggest that the occurrence of *L. monocytogenes* in RTE food products, especially RTE meat products, is a major threat to public health. Outbreaks associated with *L. monocytogenes* therefore have prompted regulatory agencies to impose stringent regulations, especially with regard to RTE foods. Since 1989 there has been a "zero tolerance" policy for *L. monocytogenes* in RTE meat products issued by the USDA. In foods in which *L. monocytogenes* is not capable of growing, Canada and European countries have instituted an action level of 100 CFU/g (Farber and Harwig, 1996; Roberts, 1994; Teufel, 1994). The compliance criteria for *L. monocytogenes* in RTE foods and the sampling scheme for these foods have been discussed (Farber and Harwig, 1996) and are listed in Table 1.1. A quantitative risk assessment for *L. monocytogenes* in RTE foods has been discussed by Rocourt et al. (2003).

1.2.2 *Incidence and prevalence of* L. monocytogenes *in RTE foods*

Listeria monocytogenes can be prevalent in a variety of RTE foods. Ready-to-eat products like vegetables, meat, poultry, seafood, and dairy have all been found to be contaminated with *L. monocytogenes* (Brackett, 1988). A survey of *L. monocytogenes* in Maryland and northern California showed 577 positive samples out of 31,705 total samples of RTE foods, including luncheon meats, deli salads, soft cheeses, bagged salads, smoked seafood, and seafood salads (Gombas et al., 2003). Seafood salads from markets in Iceland showed a 16% prevalence rate of *L. monocytogenes* (Hartemink and Georgsson, 1991). In a recent survey of salads, *L. monocytogenes* was found in 4.7% of seafood salads and 2.4% of deli salads such as coleslaw, potato salad, tuna salad, and pasta salad (Hwang and Tamplin, 2005). The use of meat, seafood, and cheese in some salads could serve as a source of *L. monocytogenes* in these salads (Smittle, 2000). *Listeria monocytogenes* was isolated from open RTE salad vegetables in retail premises in the United Kingdom (Sagoo et al., 2003). In numerous studies, samples of RTE meat products from retail stores were collected to find the prevalence of *L. monocytogenes*. The contamination level of this pathogen was 72% in corned beef and 33.8% in ham (Grau and Vanderline, 1991) and 15.5%

Table 1.1 Compliance Criteria and Sampling for *Listeria monocytogenes* (LM) in Ready-to-Eat (RTE) Foods

Category	Action Level for LM	GMP Status	Immediate Action	Sampling	Analysis
1. RTE foods causally linked to listeriosis (This list presently includes soft cheese, liver pâté, coleslaw mix with shelf-life >10 days, jellied pork tongue, RTE meats)	>0 CFU/50 g	n/a	Class I recall to retail level Consideration of public alert Appropriate follow-up at plant level	5 sample units (100 g or ml each) taken at random from each lot	5 × 10 g or 2 × 25 g analytical units are either analyzed separately or composited
2. All other RTE foods supporting growth of LM with refrigerated shelf-life >10 days	>0 CFU/25 g	n/a	Class II recall to retail level Consideration of public alert Appropriate follow-up at plant level	5 sample units (100 g or ml each) taken at random from each lot	5 × 5 g analytical units are either analyzed separately or composited
3. RTE foods supporting growth of LM with refrigerated shelf-life ≤10 days and all RTE foods not supporting growth	≤100 CFU/g	Adequate GMP	Allow sale	5 sample units (100 g or ml each) taken at random from the lot	5 × 10 g analytical units are analyzed separately Where enrichment is necessary 5 × 5 g analytical units are analyzed separately or composited
	≤100 CFU/g	Inadequate or no GMP	Consideration of Class II recall or stop sale		
	>100 CFU/g	n/a	Class II recall or stop sale		

CFU: Colony Forming Units; GMP: Good Manufacturing Practices
Source: Adapted from Farber and Harwig, 1996.

in chicken and turkey products (Rijpens et al., 1996). *Listeria monocytogenes* was isolated from 12% to 18% of precooked RTE chilled foods in England (Gilbert et al., 1989) and about one-third of RTE meat products sampled in Europe and Canada (Johnson et al., 1990).

The survival of *L. monocytogenes* in traditional Greek salads, such as fish roe salad and eggplant salad, stored at 10°C for 15 days was studied (Tassou et al., 2009). In the absence of preservatives, the organism survived well with only one log reduction, and acid adapted cells survived well even in the presence of preservatives. Various RTE foods (3,063 samples) from Florida were tested for *L. monocytogenes,* and 91 samples (2.97%) tested positive (Shen et al., 2006); 71 (78%) of these isolates exhibited multiple antibiotic resistance, and 89 (97.8%) isolates were acid resistant. These results show that RTE food isolates can adapt to various environmental stresses and thus exhibit better stress resistance.

A number of researchers have surveyed RTE foods in various nations throughout the world for incidence of *L. monocytogenes.* Minimally processed RTE fruits, vegetables, sprouts, and salads from retail outlets in Spain were surveyed for the presence of microorganisms; 0.7% of samples tested positive for *L. monocytogenes* (Abadias et al., 2008). Another survey of RTE foods (1,226 samples), including seafood, meat, dairy, and desserts obtained from the retail or food industry in Spain found *L. monocytogenes* in 20% of frozen Atlantic bonito small pies, 7.9% of smoked salmon samples, 11.1% of pork luncheon meat samples, 6.2% of frozen chicken croquettes, 16.9% of cured dried sausage samples, 12.5% of cooked ham samples, 20% of cooked turkey breast samples, 1.3% of fresh salty cheeses, and 15.1% of frozen cannelloni samples (Cabedo et al., 2008). A survey of retail outlet foods, including some RTE foods, in Japan showed the presence of *L. monocytogenes* in 5.4% of 92 smoked salmon, 3.3% of 213 raw seafood, 12.2% of 41 minced beef, 20.6% of 34 minced pork, 37% of 46 minced chicken, 25% of 16 minced pork-beef mixture, and none of the 285 vegetable samples (Inoue et al., 2000). However, another survey of various RTE foods, including raw vegetable salad, cooked salad, cooked rice, boiled noodles, bean curd, and cooked Japanese foods, revealed no presence of *L. monocytogenes* (Kaneko et al., 1999). RTE meat products in Belgian retail outlets were surveyed for *L. monocytogenes* and 13.71% of 824 raw cured meats, 4.9% of 3,405 cooked meats, 21.28% of 874 mayonnaise-based salads, and 11.7% of 786 prepared meals, tested positive for the organism (Uyttendaele et al., 1999). In this study for whole cooked meat products, more samples were positive after slicing than before slicing, indicating cross-contamination during slicing. *Listeria* spp. were recovered from about 15% of RTE food samples in Portugal (Guerra et al., 2001).

Ready-to-eat organic vegetables from retail outlets in the United Kingdom were sampled for the presence of foodborne pathogens, including

L. monocytogenes, and none was detected in 3,200 samples (Sagoo et al., 2001). In this survey only 0.5% of samples were of unsatisfactory quality due to presence of non-pathogenic *E. coli* and *Listeria* spp. Another survey of RTE organic vegetables in Northern Ireland found the absence of enteric pathogens, including *L. monocytogenes*, in 86 samples examined (McMahon and Wilson, 2001), indicating good agricultural, production, and harvest practices. Another sampling of 5,228 RTE food samples in the United Kingdom showed an incidence of >0.55% for *L. monocytogenes* and other pathogens (Meldrum et al., 2005). *L. monocytogenes* was present in 4% of samples of RTE baguettes and salads (out of 70 samples) prepared in retail delicatessens in South Africa (Christison et al., 2008). RTE street-vended foods (51 samples) in South Africa were tested for their microbiological quality and safety, and were found to be free of a number of foodborne pathogens, including *L. monocytogenes* (Mosupye and von Holy, 1999).

Sources other than foods can aid in promoting the transfer, survival, and growth of foodborne pathogens to RTE foods. Pesticides used in vegetable crop cultivation promoted the survival and growth of foodborne pathogens, including *L. monocytogenes* in some studies (Coghlan, 2000; Guan et al., 2001), while in some other studies *L. monocytogenes* did not survive in pesticides (Ng et al., 2005). Those pesticides promoting survival or growth are a concern for vegetable growers, since they can be a source of contamination of vegetables in the field. Cleaning and handling tools such as floor mops, cleaning cloths, and disposable gloves used in retail delicatessens in South Africa were sampled for the presence of bacteria, including *L. monocytogenes* (Christison et al., 2007). The organism was detected in five cleaning tool samples as well as other bacteria detected in several samples. These tools, therefore, can play a role as reservoirs for transferring contamination to RTE meats prepared in delicatessens.

Contamination of RTE products can occur from many different sources. Raw meat used for manufacturing of meat products, the processing environment, processing equipment, and the workers engaged in the manufacturing can cause contamination. RTE meat products are cooked in casings but are re-exposed to the processing environment to be packed in a final retail-packaging wrap. This procedure can lead to contamination of RTE meat products with pathogens on conveyors, meat surfaces, in condensation drippage, contaminated air filters, splashed standing water, or the workers themselves. Many RTE products are often consumed without further thorough cooking, which can lead to illness. Current efforts in meat and meat product safety have included reduction of pathogens in the raw meat during slaughtering, using treatments like carcass pasteurization, vacuum steaming, acid or water rinse, and other methods. Strategies for controlling or reducing *L. monocytogenes* in RTE foods are discussed later in this chapter.

Due to several large outbreaks that occurred in the 1980s and 1990s, the U.S. Food and Drug Administration (FDA) and the USDA-FSIS established a policy for RTE foods which mandates that the presence of *L. monocytogenes* at 0.04 CFU/g in a RTE food renders the product adulterated (Smoot and Pierson, 1997). Since then the RTE food industry has taken stringent measures to control *L. monocytogenes* in their products. However, *L. monocytogenes* contamination continues to occur, probably attributable to its ubiquitous nature. Surveillance and monitoring of RTE foods by the USDA and FDA have indicated that as many as 5% of some of the RTE foods such as sliced luncheon meats and prepared deli style salads show *L. monocytogenes* contamination (Hitchins, 1996; Levine et al., 2001). The incidence of *L. monocytogenes* in RTE foods can range from 1% to 10% (Farber and Harwig, 1996).

1.2.3 *Strategies for controlling* L. monocytogenes *in RTE foods*

In May 1999, the FSIS announced strategies for controlling *L. monocytogenes* in RTE food products (FSIS, 1999). These include mandatory implementation of Hazard Analysis and Critical Control Points (HACCP) at various processing steps to reduce the number of pathogens in the manufacturing environment, end-product testing, and educating consumers on the risk of listeriosis and prevention of *L. monocytogenes* growth. For long-term goals, the FSIS conducted studies of *L. monocytogenes* post-production growth and risk assessment in RTE meat products (FSIS, 1999). Based on the results of risk assessment conducted by FSIS with FDA, the agency acknowledged three other approaches for controlling *Listeria monocytogenes* in RTE meat products. The first alternative was for the establishments to check for HACCP plans, go through a lethality treatment, and incorporate growth-inhibiting agents and processes for *Listeria* in their products. The second alternative was that the establishment would employ a lethality treatment and incorporate a growth-inhibiting agent or a process to address *Listeria monocytogenes*. The third alternative was for the establishment to rely on sanitation Standard Operating Procedures (SOPs) for processing environments, particularly food contact surfaces, to be free of *Listeria*.

Listeria monocytogenes is a difficult organism to control because of its hardy characteristics. It has a relatively high tolerance to heat compared to other non-spore-forming organisms, can survive and multiply at refrigeration temperatures in the presence or absence of oxygen, withstand a wide pH range (pH 4.1–9.6) and concentrations of salt up to 12% to 13% (Lou and Yousef, 1999). Some strains can grow at a water activity (a_w) as low as 0.9 and at pH value as low as 4.4 (Farber and Peterkin, 1991; Miller, 1992; Walker et al., 1990). Also, *Listeria* can form biofilms on various food and food contact surfaces. Since *L. monocytogenes* is able to grow

at refrigeration temperatures, if RTE foods are contaminated, it can grow to dangerous levels during storage and transportation from production facility to retail distributors, even at refrigeration temperatures.

On RTE fresh fruits and vegetables, *L. monocytogenes* can be removed using washing treatments with or without sanitizers. Washing fresh produce using cold chlorinated water is a common practice used by the industry. Washing, using solutions of natural antimicrobials such as plant extracts, could prove useful and is an area that needs further investigation. Delaquis et al. (2002) utilized warm (47°C) and cold (4°C) chlorinated water (100 mg/l chlorine) for washing cut iceberg lettuce which was stored at 1°C or 10°C. Cold chlorinated water was more effective against *L. monocytogenes* than was warm chlorinated water, which permitted growth of the organism on lettuce stored at 10°C. Washing treatments on whole cantaloupes using 1000 ppm chlorine or 5% hydrogen peroxide for 2 minutes and storing at 4°C for 15 days reduced the pathogen population by 3.5 logs (Ukuku and Fett, 2002). On fresh cut pieces from the sanitized cantaloupes, *L. moncytogenes* survived but did not grow during 15 days storage at 4°C.

The growth of *L. monocytogenes* in RTE meat products can be controlled by changing internal parameters of food products, such as reduction of a_w and pH with direct acidification, incorporation of antimicrobials in meat products, and modified atmospheric packaging (Nilsson et al., 1997; Pothuri, 1995; Zeitoun and Debevere, 1991). In the processed meats, reduction procedures include incorporating antimicrobials and post-packaging treatments of RTE meat products with microwave (Schalch et al., 1995), steam (Cygnarowicz-Provost, 1994), or hot water (Cooksey et al., 1993a, 1993b). Antimicrobials of natural origin or chemical preservatives can be used and can be added as direct ingredients in the formulation. Bacteriocins are compounds produced by microorganisms that have antimicrobial activity against closely related species. Pediocin and nisin are examples of commonly used bacteriocins. Compounds like organic acids and their salts have shown antimicrobial activity against common foodborne pathogens. These compounds are classified as Generally Recognized As Safe (GRAS) when added to RTE meat products.

Research undertaken within the past decade has indicated that bactericidal compounds such as salts of organic acids and bacteriocins can control *L. monocytogenes* in meat products. In many instances combination treatments work better than individual treatments. Sodium diacetate used as a dipping treatment inhibited growth of *L. monocytogenes* on sliced pork bologna stored at 4°C for 120 days (Samelis et al., 2001). Sodium lactate used alone or in combination with sodium acetate, sodium diacetate, or glucono-δ-lactone inhibited growth of *L. monocytogenes* on frankfurters during 120 days of storage (Samelis et al., 2002). Heating the

frankfurters further reduced the organism population. A combination of sodium lactate and sodium diacetate was bacteriocidal against *L. monocytogenes* strain Scott A and bacteriostatic against a cocktail of six strains of *L. monocytogenes* (Mbandi and Shelef, 2002). Chen et al. (2002) showed that 3000 and 6000 AU of pediocin reduced the population of *L. monocytogenes* by 1.5 and 2.0 logs, respectively, on frankfurters stored at 4°C for up to 12 weeks. On beef franks, a combination of sodium lactate, sodium diacetate, and pediocin used as a dipping treatment reduced the populations of *L. monocytogenes* single strains as well as cocktail by 1 to 1.5 logs and 1.5 to 2.5 logs, respectively, after 2 and 3 weeks of storage at 4°C (Uhart et al., 2004).

Sodium diacetate as individual treatment or in combination with potassium benzoate and/or sodium lactate as dip treatments inhibited *L. monocytogenes* on frankfurters (Lu et al., 2005). Acetic acid, sodium diacetate, and potassium benzoate inhibited *L. monocytogenes* growth on pork bologna for 120 days of storage (Samelis et al., 2001). Seman et al. (2008) assessed the effects and interactions of sodium benzoate, sodium diacetate, and sodium chloride for inhibiting *L. monocytogenes* in RTE meat products, and sodium benzoate was more effective when combined with sodium diacetate and sodium chloride. These authors concluded that low moisture products such as bologna or wieners could have a shelf life longer than 18 weeks, if they were formulated with 0.1% sodium benzoate and 0.1% sodium diacetate.

A number of studies have investigated the effectiveness of natural compounds derived from plants or microorganisms against *L. monocytogenes* in various non-meat RTE foods. Citron essential oil was effective against *L. monocytogenes* in fruit-based salads, reducing the population by >4 logs in 3 days, and below detection in about 7 days (Belletti et al., 2008). The antimicrobial activity of some plant extracts and essential oils used as flavor ingredients in confectionary products stored at 7°C and 20°C for 9 days were studied; strawberry flavor in chocolate inhibited *L. monocytogenes* (Kotzekidou et al., 2008). A combination of nisin and ALTA™2341 (pediocin-like product) reduced *L. monocytogenes* on smoked salmon packaged under vacuum or 100% CO_2 (Szabo and Cahill, 1999). Lactic acid bacteria were inhibitory to *L. monocytogenes* on vacuum-packed cold smoked salmon stored at 6°C or 8°C for 35 days (Mejlholm and Dalgaard, 2007). Green tea powder added to Oriental-style rice cakes stored at 22°C ± 2°C was more effective than rosemary leaf powder against *L. monocytogenes* (Lee et al., 2009).

Since RTE meat and poultry products have been involved in a number of outbreaks with *L. monocytogenes*, antimicrobial interventions on the surface of these products have been researched extensively. Nisin incorporated at 2500 IU/ml into methyl cellulose or hydroxypropyl methyl

cellulose solutions made into films inactivated *L. monocytogenes* on the surface of hot dogs (Franklin et al., 2004). Alginate films incorporated with essential oils of Spanish oregano, Chinese cinnamon, and winter savory and pretreated with 20% $CaCl_2$ were effective against *L. monocytogenes* on bologna stored at 4°C; however, these films were not effective on ham (Oussalah et al., 2007). A combination of nisin with grapeseed extract inactivated *L. monocytogenes* on turkey frankfurters at 4°C and 10°C; however, neither antimicrobial treated alone was effective (Sivarooban et al., 2007). Edible zein film coatings containing nisin and calcium propionate reduced the population of *L. monocytogenes* on RTE chicken by 5 logs at 8°C and to undetectable levels at 4°C in 24 days (Janes et al., 2002).

Ready-to-eat meats can also be treated using physical means for inactivating *L. monocytogenes.* The physical methods employed include heat, high pressure, drying, smoking, and irradiation. High hydrostatic pressure treatment (400 MPa, 17°C, 10 min) of sliced cooked ham, in combination with 1.8% potassium lactate followed by storage at 1°C was effective in inactivating *L. monocytogenes* (Aymerich et al., 2005). Ionizing radiation alone and in combination with sodium diacetate and potassium lactate was effective against RTE frankfurter on a roll product (Sommers and Boyd, 2006). Foong et al. (2004) treated RTE meats using irradiation against *L. monocytogenes.* To obtain 3- and 5-log reductions, respectively, dosages of 1.5 and 2.5 kGy were needed for bologna, roast beef, and smoked turkey, and 2.0 and 3.0 kGy were needed for frankfurters and ham. Combining irradiation with antimicrobials may help reduce the high dose and also prevent the growth of *L. monocytogenes* during storage. Combinations of irradiation at 1- or 2-kGy doses with potassium benzoate and sodium lactate, or sodium lactate and sodium diacetate, or a combination of all three salts inhibited growth of *L. monocytogenes* on RTE turkey breast rolls stored at 4°C for 42 days (Zhu et al., 2009). Combination treatments help in maintaining sensory attributes of the product by permitting use of lower levels of antimicrobials or lower intensity of the physical treatment than needed, when these antimicrobials or physical treatments are used alone as a single treatment.

Using post-package pasteurization of RTE deli meats including turkey, ham, and roast beef by submersion heating for 2 to 10 minutes at 195°F to 205°F, 2- to 4-log reductions in *L. monocytogenes* populations were achieved (Muriana et al., 2002). An integrated pasteurization-packaging system using 121°C for 1.5 seconds, followed by vacuum sealing of the top films of food packages, rendered a 3-log reduction in *L. monocytogenes* population on cooked franks (Murphy et al., 2005). Processing bologna using a cooking cooling cycle commonly used by the industry resulted in a 5-log reduction of *L. monocytogenes* (Sallami et al., 2006). Ozone was found to be of limited effectiveness against

L. monocytogenes on RTE cured ham (Julson et al., 2001). RTE meat products such as summer sausage, smoked cured beef, beef jerky, and snack stick pork rind were made using drying, fermentation, and/or smoking, inoculated with *L. monocytogenes* cocktail, repackaged under vacuum or air and stored at 21°C or 5°C for 11 weeks (Ingham et al., 2004). There was a decrease in the population of the pathogen from 0.8 to 3.3 logs in various products under different conditions, indicating that these processes combined with storage for at least 1 week would be an effective post-lethality strategy for controlling *L. monocytogenes* in some of these products.

Al-Holy et al. (2004) employed radio frequency heating along with nisin to inactivate *L. innocua* (a surrogate for *L. monocytogenes*) on sturgeon caviar, and the combination treatment at 65°C was effective; no survivors were detected. Pulsed electric field treatment (30–50 kV/cm) followed by exposure to nisin (10–100 IU) reduced *L. innocua* (a surrogate for *L. monocytogenes*) population in skim milk by 2 to 3.8 logs (Calderón-Miranda et al., 1999). Pulsed electric field treatment alone, however, was not effective against *L. monocytogenes* in skim milk (Fleischman et al., 2004). Nisin in combination with moderate heat (60°C for 5 min or 65°C for 2 min) reduced *L. monocytogenes* population by 3 to 5 logs in cold pack lobster meat; either treatment alone had only 1- to 3-log reductions (Budu-Amoako et al., 1999). Mild heating (60°C for 3 min) with nisin reduced *L. monocytogenes* on sturgeon caviar stored for 28 days at 4°C to below detection; no synergy was observed between nisin, lactic acid, or chlorous acid (Al-Holy et al., 2005).

1.3 Salmonella enterica

The *Salmonella* genus includes rod-shaped, Gram-negative, non-spore-forming, predominantly motile enteric (intestinal) bacteria. The bacteria range in diameter from 0.7 to 1.5 μm, and in lengths from 2 to 5 μm, with peritrichous flagella. These bacteria obtain their energy from oxidation-reduction reactions using organic substrates and are facultative anaerobes. Most *Salmonella* species produce hydrogen sulfide, are unable to ferment lactose, and can readily be detected by growing on media containing ferrous sulfate (Giannella, 1996).

Salmonella has been known to cause illness for over 100 years. The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. Although Theobald Smith was the actual discoverer of the type bacterium (*Salmonella enterica* var. choleraesuis) in 1885, Dr. Salmon was the administrator of the research program; hence the organism was named after him (USFDA, 2007). Smith and Salmon had been investigating the cause of common hog cholera and suggested

Salmonella as the causal agent. However, it was later found out that this organism (now known as *Salmonella enterica*) rarely caused enteric symptoms in pigs (Todar, 2008). *Salmonella* are closely related to *Escherichia coli* (~ 90% homology at DNA level) and are found in warm- and cold-blooded animals, in humans, and in nonliving habitats. They cause illnesses in humans such as typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis (Ryan and Ray, 2004).

There are more than 2,500 serotypes of *Salmonella*, based upon the somatic or cell wall antigens (O-antigen), flagellar antigens (H-antigen), and surface or envelope antigens (Todar, 2008). Two of these serotypes, namely, *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium are the most common in the United States and account for 50% of all human infections (CDC 2005a; Vugia et al., 2004). The infectious dose of *Salmonella* is small, probably ranging from 15 to 20 cells, and most strains cause gastroenteritis with an incubation period of 6 to 72 hours (CDC, 2001). The initial stage of infection includes symptoms such as nausea, cramping, and vomiting, followed by a second stage typified by severe diarrhea and cramping lasting for 2 to 7 days.

Salmonella causes one of the most common enteric infections in the United States, salmonellosis, which is the second most common bacterial foodborne illness reported (second to *Campylobacter* infection) (CDC, 2005b). However, the actual number of infections may be 30 or more times greater since many cases with mild symptoms are not diagnosed or reported (Mead et al., 1999). According to the CDC estimates, 1.4 million cases occur annually in the United States, amounting to about 30,000 confirmed cases of salmonellosis each year. *Salmonella* illnesses result in approximately 600 deaths annually, which accounts for 31% of all food-related deaths (CDC, 2005b). The incidence of salmonellosis appears to be rising both in the United States and in other industrialized nations. In 2005, more than 36,000 cases were reported from public health laboratories across the nation, which represented a 12% decrease compared to the previous decade, but a 1.5% increase compared to those reported in 2004 (CDC, 2007b). Isolation of *S.* Enteritidis from humans has shown that *Salmonella* infections have increased dramatically (six-fold or more) in the past decade, particularly in the northeast United States and is spreading south and west, with sporadic outbreaks in other regions (U.S. Food and Drug Administration, 2009).

1.3.1 *Outbreaks associated with* Salmonella *in RTE foods*

Salmonella has been linked to several foodborne outbreaks associated with RTE foods in the past two decades. RTE foods most commonly associated

with *Salmonella* include poultry products, egg products, milk and dairy products, seafood, yeast, coconut, sauces and salad dressings, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, chocolate, and fresh produce (USFDA, 2009).

Foodborne outbreaks of *Salmonella* have been a major concern in RTE meat and poultry products. In June 2007, a multistate outbreak of *Salmonella* infections associated with frozen pot pies occurred in the United States (CDC, 2007a). The outbreak involved 401 cases of salmonellosis in 41 states; 32% of affected people were hospitalized. The outbreak strain was isolated from 13 samples of unopened Banquet pot pies collected from the homes of patients. Further investigations revealed that 77% of patients failed to cook the product properly due to confusion regarding microwaving instructions. In February 1995, 93 cases of salmonellosis associated with beef jerky were identified by the New Mexico Department of Health (NMDOH) (CDC, 1995b). The *Salmonella* serotypes isolated from patients were *Salmonella* Typhimurium (31 persons), *Salmonella* Montevideo (12 persons), and *Salmonella* Kentucky (11 persons).

While meat and poultry products top the list of RTE foods associated with *Salmonella* outbreaks, other RTE foods have also been implicated in *Salmonella* outbreaks. Toasted oats cereal was implicated in a multistate outbreak of *Salmonella* serotype Agona in April and May 1998 (Martin and Bean, 1995). A total of 209 cases with at least 47 hospitalizations were reported. *Salmonella* Agona is an uncommon serotype, accounting for approximately 1.5% of human isolates reported to the Public Health Laboratory Information System (PHLIS) (Martin and Bean, 1995). Like most other *Salmonella* serotypes, *S.* Agona is found in a variety of animal reservoirs, including poultry, cattle, pigs, and animal feed. This outbreak represented the first commercial cereal product to have been implicated in a *Salmonella* outbreak, although an infant cereal product was implicated in an outbreak of *Salmonella* Senftenberg in the United Kingdom (Rushdy et al., 1988). *Salmonella* spp. are relatively resistant to desiccation and can survive for long periods in dry environments such as cereal (Mitscherlich and Marth, 1984). Other outbreaks of this serotype have been attributed to dried milk and to a commercial peanut-flavored snack (Sramova et al., 1991). Another outbreak of *Salmonella* Enteritidis occurred in 1994 in ice cream which had 80 confirmed cases (CDC, 1994).

In recent years, fresh produce has been repeatedly implicated as a vehicle for foodborne illnesses due to *Salmonella*. Together with *E. coli* O157:H7, *S. enterica* is responsible for approximately 61% of all produce-associated illnesses (Olsen et al., 2000). Green onions, lettuce, spinach, cantaloupes, tomatoes, cabbage, strawberries, raspberries (Beuchat, 1996), alfalfa sprouts, parsley (Harris et al., 2001), and tree nuts (CDC, 2009b; USFDA,

2004) have been implicated as vehicles of bacterial infections. According to the Center for Science in the Public Interest (CSPI, 2009), alfalfa sprouts have been implicated in more than 20 *Salmonella* outbreaks since 1995. An outbreak of *S. enterica* was associated with irrigation water used to produce alfalfa sprouts in California (Winthrop et al., 2003). *Salmonella* Saintpaul was identified as the causative agent for 228 cases of foodborne illnesses that recently occurred in Nebraska and 13 other states, due to consumption of alfalfa sprouts between January and May 2009. The outbreak was traced to alfalfa sprouts grown at multiple facilities that used seeds possibly originating from a common seed producer, identified as Caudill (CDC, 2009b). In April 2008, another large outbreak of *Salmonella* Saintpaul was initially linked to raw tomatoes in New Mexico and Texas, when CDC issued warnings against consumption of raw tomatoes, including round red and Roma tomatoes (CDC, 2008). Over the summer, the outbreak spread to 43 states, the District of Columbia, and Canada. By August, more than 1,400 people were confirmed ill. However, as the outbreak continued to spread, the advisory on tomatoes was lifted and issued for Mexican-grown raw jalapeño and serrano peppers. On July 30, the FDA confirmed the presence of *Salmonella* Saintpaul at a farm in Mexico, both in irrigation water and on produce.

Between October 2006 and January 2007, an outbreak of 26 cases of *Salmonella* Litchfield infection in the states of Western Australia and Queensland was linked to consumption of papaya (Gibbs et al., 2009). Inspection of two of the three farms in Western Australia that supplied the contaminated papaya revealed that *Salmonella* Litchfield was not detected in papaya samples, fungal sprays, or water samples from the farms; however, other serotypes of *Salmonella* were detected in untreated river water used for washing papaya at one of the farms.

The most recent peanut butter outbreak of *S. enterica* spanned 47 states and involved more than 500 cases and 8 fatalities (CDC, 2009c). Inadvertent moisture in the production facility possibly allowed the growth of dormant *Salmonella* organisms that were likely present in raw peanuts or peanut dust. A leaky roof in the plant during a rainstorm and a faulty sprinkler system were suspected to have contributed to the moisture. An earlier outbreak in 2006–2007 involved peanut butter and *S. enterica* (CDC 2007b). Yet another *Salmonella* outbreak was definitively linked to raw almonds, leading to the recall of roughly 18 million pounds of raw almonds produced by Paramount Farms (USFDA, 2004). Environmental investigations into the outbreak revealed that *Salmonella* was present at the farm when three samples from two huller-shellers that supplied the farm during the period of interest tested positive for *Salmonella* contamination.

1.3.2 *Incidence and prevalence of* Salmonella *in RTE foods*

Ready-to-eat products such as salad vegetables, leafy greens, meat, poultry, seafood, dairy, and tree nuts, along with herbs, spices, and dried seeds, have all been found to be contaminated with *Salmonella*. The organism is more prevalent in RTE products of animal origin, possibly due to its enteric nature and the ability to survive extreme environmental conditions. Prevalence of *Salmonella* was estimated in different types of RTE food products of animal origin in Catalonia, Spain (Cabedo et al., 2008). A total of 1,379 samples consisting of 187 RTE fish products and 569 RTE meat products, 484 RTE dairy products, and 139 RTE dishes and desserts were collected and analyzed for the presence of *Salmonella*. The organism was isolated from 1.2% of smoked salmon samples, 1.5% of frozen chicken croquettes, 2% of cooked ham samples, and 11.1% of cured dried sausage samples. Between 1990 and 1999, FSIS conducted microbiological testing for nine different categories of RTE meat and poultry products that were produced at approximately 1,800 federally inspected production facilities (Levine et al., 2001). The cumulative *Salmonella* prevalence over the 10-year period was found to be 0.31% in jerky; 0.10% in cooked, uncured poultry products; 0.07% in large-diameter cooked sausages; 0.20% in small-diameter cooked sausages; 0.22% in cooked beef, roast beef, and cooked corned beef; 0.05% in salads, spreads, and pâtés; and 0.22% in sliced ham and luncheon meat. For dry and semidry fermented sausages, the cumulative 3-year *Salmonella* prevalence was 1.43%.

Recent outbreaks of salmonellosis due to consumption of products from plant origin have highlighted the relevance of sources of infections other than animal origin (e.g., egg, poultry, meat). An international outbreak of multi-drug-resistant *S.* Typhimurium DT 104 was correlated to the consumption of halvah, an Asian sweet confection made from sesame seeds. In a follow-up study conducted by Brockmann et al. (2004), several sesame seed products were examined for the occurrence of *Salmonella*. Of the 117 RTE food products made from sesame seeds, salmonellae were isolated from 11 (9.4%) samples (Brockmann et al., 2004). In addition to finding *S.* Typhimurium DT 104 in the halvah involved in the outbreak, *S.* Offa, *S.* Tennessee, and *S.* Poona were also isolated from sesame paste (tahini) and sesame seed sold for raw consumption in cereals. Due to the high desiccation tolerance of *Salmonella*, dried herbs and spices have also been found to be contaminated with this organism. The FDA has noted an increase in the number of recalls of dried spices due to bacterial contamination (Viji et al., 2006). Accordingly, Viji et al. (2006) reviewed spice recalls in the United States that took place between 1970 and 2003. Of the 21 recalls involving 12 spice types contaminated with bacterial pathogens,

20 recalled spices contained *Salmonella*, with paprika being the most common spice involved in the recalls.

A more recent study carried out by the Health Protection Agency and the Local Authorities Co-ordinators of Regulatory Services (LACORS) (2009) revealed the presence of *Salmonella* in a small number of RTE dried seed samples. Between October 2007 and March 2008, 3,735 samples of RTE seeds of different varieties (alfalfa, hemp, linseed, melon, poppy, pumpkin, sesame, sunflower, other [watermelon, celery] and mixed seeds) were collected from retail premises including supermarkets, health food shops, convenience shops, and market stalls. *Salmonella* was detected in 0.6% of samples collected; samples included sesame seeds, linseed, sunflower seeds, alfalfa seeds, melon seeds, and mixed seeds. The most frequently contaminated seed type was melon seeds (8.5% contained *Salmonella*).

Consumer demand for fresh fruits and vegetables and for convenience foods has increased in the past decade, causing an expansion of the minimally processed vegetables (MPVs) market. *Salmonella* is one of the more common pathogenic microorganisms that can be transmitted to humans by these products. Fröder et al. (2007) evaluated the microbial quality of a variety of MPVs. A total of 133 samples of minimally processed leafy salads were tested for the presence of *Salmonella* from retailers in the city of São Paulo, Brazil. Salmonella was detected in 4 (3%) of the 133 samples. Similarly, a study of retail, bagged, prepared RTE salad vegetables was undertaken by Sagoo et al. (2003) to determine the microbiological quality of these vegetables. Examination of 3,852 samples of the salad vegetables revealed that 6 (0.2%) samples were of unacceptable microbiological quality because of the presence of *Salmonella* (*Salmonella* Newport PT33 [1 sample], *Salmonella* Umbilo [3 samples], and *Salmonella* Durban [1 sample]). Prevalence of *Salmonella* on fresh produce collected from the southern United States was evaluated by Johnston et al. (2005). A total of 398 produce samples (leafy greens, herbs, and cantaloupe) were collected. The prevalence of *Salmonella* for all samples was 0.7% (3 of 398).

1.3.3 *Strategies for controlling* Salmonella *in RTE foods*

Salmonella is associated with a number of foods. Due to its enteric nature, meat (cattle, pigs, goats, chickens, etc.) may be contaminated from the intestinal contents during evisceration of animals, during washing, and during post-harvest processing. However, proper cooking of the meat and meat products will generally eliminate the contamination. Therefore, control of *Salmonella* focuses on adequate cooking of potentially contaminated foods. The multistate outbreak of *Salmonella* infections associated with inadequately cooked frozen pot pies emphasizes the need to thoroughly cook non-RTE frozen foods. Additionally, these products need to

be clearly labeled, with cooking instructions validated to account for variability in microwave wattage and common misconceptions among consumers regarding the nature of non-RTE foods.

Ready-to-eat foods such as cream, mayonnaise, and creamed foods can support multiplication of *Salmonella*. Additionally, many RTE foods are either consumed raw or are minimally processed. Vegetables and fruits may carry *Salmonella* when contaminated with fecal matter or when washed with polluted water. Minimally processed cut and packaged salad is exposed to a range of conditions during growth, harvest, preparation, and distribution, and these conditions may increase the potential for microbial contamination. It is therefore essential to prevent cross-contamination by implementation of good agricultural practices and good hygiene practices from farm to fork.

Additionally, *Salmonella* is extremely tolerant to various growth conditions. It can grow over a temperature range of 7°C to 46°C, survive in water activity as low as 0.94, and in pH ranging from 4.4 to 9.4 (FSIS, 2008). The desiccation resistance of *Salmonella*, along with its ability to survive varying temperature and pH conditions, plays a role in the survival of this organism in RTE products. Therefore, conditions recommended for the prevention of bacterial growth during production of certain RTE meat products such as jerky include rapid drying at high temperatures (i.e., initial drying temperature greater than 155°F [68.3°C] for 4 hours, then greater than 140°F [60°C] for an additional 4 hours) and decreased water activity (i.e., $a_w = 0.86$) (Holley, 1985a, 1985b). *Salmonella* infection can thus also be prevented by avoiding multiplication of the organism in food (constant storage at 4°C), and by using pasteurized and sterilized products (fruit juices, milk, and milk products).

FSIS is the public health regulatory agency in the USDA that is responsible for the safety of the United States' commercial supply of meat, poultry, and egg products. As part of its responsibility, the FSIS issued the "Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems, Final Rule" in 1996. This rule sets performance standards for establishments that slaughter a selective class of food animals, or those that produce selected classes of raw ground products, to verify that industry systems are effective in controlling the contamination of raw meat and poultry products with disease-causing bacteria, such as *Salmonella*. FSIS requires all plants to reduce bacteria by means of the PR/HACCP system. FSIS has also taken steps at addressing the problems of *Salmonella* contamination on meat and poultry products. Since 1983, FSIS has been conducting regulatory microbiological testing programs on RTE meat and poultry products. These programs are designed to evolve continuously in response to public health concerns and provide an overall indication of trends.

1.4 Escherichia coli *O157:H7*

Escherichia coli, considered to be part of the normal micro-flora of the intestinal tract of humans and other warm-blooded animals (Drasar and Hill, 1974), was first described by Theodor Escherich in 1885 (Escherich, 1988). Generally, *E. coli* strains that colonize the human intestine are harmless. However, they may become pathogenic and have the potential to cause infection among immunocompromised individuals or when the integrity of the defense barrier system of the intestinal mucosa is compromised (Nataro and Kaper, 1998). Over the years some *E. coli* strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system, even in healthy individuals (Nataro and Kaper, 1998). The diarrheagenic *E. coli* that have been associated with foodborne illnesses are grouped into five categories: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), and enterohemorrhagic *E. coli* (EHEC). *Escherichia coli* O157:H7 belongs to the EHEC group of *E. coli* that has been associated with foodborne illnesses. It is one of the most important strains belonging to the EHEC group and has been commonly associated with hemorrhagic colitis and hemolytic uremic syndrome around the world.

Escherichia coli O157:H7 was first isolated in 1975 from a woman in California diagnosed with severe bloody diarrhea (Riley et al., 1983). It was first recognized as an important pathogen in 1982 following two foodborne outbreaks of hemorrhagic colitis (Riley et al., 1983; Wells et al., 1983). In 1983, Karmali et al. (1983) reported an association between infection with Shiga toxin–producing *E. coli* and hemolytic uremic syndrome. In recognition of its distinct clinical manifestations, *E. coli* O157:H7 became the first of several strains to be referred to as EHEC, which are now believed to account for more than 90% of all cases of hemolytic uremic syndrome in industrial countries (Scotland et al., 1985). *Escherichia coli* O157:H7 is genetically very closely related to *E. coli* O55:H7, an EPEC strain associated with infantile diarrhea (Whittman et al., 1993). It can adhere to epithelial cells and produce the characteristic "attaching and effacing" lesions (Nataro and Kaper, 1998) similar to the EPEC strain. According to LeClerc et al. (1996), *E. coli* O157:H7 may be particularly adept at incorporating foreign DNA material due to its intrinsically high rate of defects in the DNA repair mechanisms.

Several factors have been associated with the virulence of *E. coli* O157:H7; one factor is its ability to produce one or more Shiga toxins. It was demonstrated in 1983 that the genes controlling the production of the two toxins are bacteriophage encoded (O'Brien et al., 1984; Scotland et al., 1983; Smith et al., 1983). It is believed that the bacteriophage was acquired by the *E. coli* O157:H7 strains directly or indirectly from *Shigella*

(Buchanan and Doyle, 1997). Production of Shiga toxins in itself is not sufficient to cause disease. *E. coli* O157:H7 has other characteristics that help make it virulent and deadly. Adhesion to the epithelial cells lining the intestinal tract may be one important aspect of the organism's pathogenic potential (Griffin, 1995). Other factors thought to contribute to the virulence of *E. coli* O157:H7 include a 60MDa virulence plasmid (pO157) and the locus of enterocyte effacement (LEE) (Mead and Griffin, 1998). The involvement of the 60MDa plasmid in adherence has been suggested, but the reports on its exact role are conflicting (Karch et al., 1987; Toth et al., 1991; Tzipori et al., 1987). It has also been suggested that the pO157 encodes a hemolysin that, in concert with specialized transport systems, may allow *E. coli* O157:H7 to use iron from blood released into the intestine (Law and Kelly, 1995).

1.4.1 *Outbreaks associated with* E. coli *O157:H7 in RTE foods*

In the 10 years following the 1982 outbreak, approximately 30 *E. coli* O157:H7 outbreaks were recorded in the United States (Griffin and Tauxe, 1991). The actual number that occurred is probably much higher because *E. coli* O157:H7 infections did not become a reportable disease (required to be reported to public health authorities) until 1987 (Keene et al., 1991). As a result, only the most geographically concentrated outbreaks would have garnered enough attention to prompt further investigation (Keene et al., 1991). It is important to note that only about 10% of infections occur in outbreaks; the rest are sporadic. It is estimated that *E. coli* O157:H7 causes approximately 73,000 illnesses in the United States each year. The Centers for Disease Control and Prevention (CDC) has estimated that 85% of *E. coli* O157:H7 infections are foodborne in origin (Mead et al., 1999). Between 1982 and 2002, of the 350 outbreaks caused by *E. coli* O157:H7, 52% were caused by foodborne sources (Rangel et al., 2005). Although undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks, RTE foods and produce such as sausages, dried (uncooked) salami (Alexander et al., 1994), non-pasteurized milk and cheese, yogurt, non-pasteurized apple juice and cider (Cody et al., 1999), orange juice, alfalfa and radish sprouts (Breuer et al., 2001), lettuce, and spinach (Friedman et al., 1999) have also been implicated in several *E. coli* O157:H7 outbreaks.

Due to the association of *E. coli* O157:H7 with cattle, milk and milk products have been implicated in several foodborne disease outbreaks associated with this organism. Seven outbreaks of *E. coli* O157:H7 have been associated with dairy products, including four from consuming raw milk. The others involved cheese curds and butter made from raw milk,

and commercial ice cream bars (possibly due to cross-contamination) (Rangel et al., 2005).

Beef jerky and other processed meat products, including fermented salami and sausages which are considered to be RTE and, therefore, are expected to be pathogen-free, have also been implicated in foodborne outbreaks of *E. coli* O157:H7 (Alexander et al., 1994; Williams et al., 2000). In the 1994 foodborne illness outbreak in Washington and California, 20 laboratory confirmed cases of *E. coli* O157:H7 were linked to the consumption of dry cured sausage. An outbreak of *E. coli* O157:H7 infection due to consumption of Genoa salami was identified in the spring of 1998 in southern Ontario. These products are made from raw ground meat, usually beef and pork, and are preserved through fermentation and drying, with the addition of salt and spices. It was a common belief among the manufacturers that pathogens like *E. coli* were unable to grow in foods processed under low pH, low water activity, and high salinity conditions and that fermented sausages, therefore, did not require cooking and were RTE with no further preparation.

Foodborne outbreaks of *E. coli* O157:H7 associated with fresh produce were first reported in 1991 (Rangel et al., 2005) and have become increasingly common over the past two decades. The increased consumption of fresh fruits and vegetables in the United States appears to correlate with increased produce-associated outbreaks (Rangel et al., 2005). Since 1995, there have been 22 outbreaks of *E. coli* O157:H7 associated with fresh lettuce or spinach (USFDA, 2001). According to surveillance results for *E. coli* O157 outbreaks reported to CDC between 1982 and 2002, fresh produce accounted for 38 of 183 (21%) foodborne outbreaks and 34% of 5,269 cases related to foodborne outbreaks (Rangel et al., 2005). The median number of cases in produce-associated outbreaks was significantly greater than that of ground beef-associated outbreaks (20 vs. 8 at $p < 0.001$) (Rangel et al., 2005). Fresh produce types most commonly associated with these outbreaks included lettuce (34%), apple cider or apple juice (18%), salad (16%), coleslaw (11%), melons (11%), sprouts (8%), and grapes (3%). These outbreaks most commonly occurred in restaurants (39%), out of which 47% were reported to be due to cross-contamination during food preparation. However, more than half (53%) of the produce-associated outbreaks did not involve cross-contamination in the kitchen. The outbreaks were due to produce already contaminated with *E. coli* O157 before purchase and included 7 outbreaks associated with apple cider or apple juice, 7 of 10 lettuce-associated outbreaks, 3 of 4 coleslaw-associated outbreaks, and 3 alfalfa sprout or clover sprout associated outbreaks (Ackers et al., 1998; Besser et al., 1993; Cody et al., 1999; Hilborn et al., 1999; Mahon et al., 1997). These produce items may have become contaminated in the field from manure or contaminated irrigation water; during processing due

to contaminated wash water or ice, contaminated equipment, or poor handling practices; during transport; or through contaminated storage equipment.

In 1996, a large *E. coli* O157 outbreak, attributed to the consumption of commercial unpasteurized apple juice, occurred in three western states and British Columbia, involving 70 illnesses, mostly children. More than 30% of patients were hospitalized and hemolytic uremic syndrome developed in 20% resulting in death of one child (Cody et al., 1999). Since 1998, only two outbreaks due to unpasteurized apple cider have been reported, one at a local fair and one from locally produced cider. In September 2006, there was an outbreak of foodborne illness caused by *E. coli* O157:H7 in 26 U.S. states which was traced to organic bagged fresh spinach (CDC, 2006; FDA, 2007). The outbreak resulted in 204 cases of illness, including 31 cases involving hemolytic uremic syndrome, 104 hospitalizations, and 3 deaths. Investigations by the CDC and a joint report by the California Department of Health Services (CDHS, 2007) and the FDA concluded that the probable source of the outbreak was a cattle ranch that had leased land to a spinach grower. Although it could not be definitively decided how the spinach became contaminated, the presence of wild pigs on the ranch and the close proximity of surface waterways to irrigation wells were considered "potential environmental risk factors." The report also noted that flaws in the spinach producer's transportation and processing systems could have further spread contamination. A few months later, following this outbreak, another outbreak implicated bagged lettuce in fast-food restaurants (FDA, 2006a, 2006b, 2006c), thus raising concerns about the microbiological quality of RTE produce.

1.4.2 *Incidence and prevalence of* E. coli *O157:H7 in RTE foods*

Although *E. coli* O157:H7 is most commonly associated with undercooked or raw hamburger (ground beef), RTE foods and produce including leafy greens, alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, raw or unpasteurized milk, and cheese curds have also been associated with the organism. A survey of the prevalence of *E. coli* O157 in raw meats, raw cow's milk, and raw-milk cheeses was conducted in southeast Scotland over a 2-year period starting in April 1997. *Escherichia coli* O157 was isolated from beef sausage and beef burger produced by the same retail butcher shop. Cooley et al. (2007) described the incidence of *E. coli* O157 in a major produce production region in California following the produce-associated outbreaks between 2002 and 2006. Approximately 1,000 *E. coli* O157 isolates obtained from cultures of more than 100 individual samples were typed using Multi-Locus Variable-number-tandem-repeat Analysis

(MLVA) to identify the potential fate and transport of *E. coli* O157 in this region. The organism was isolated at least once from 15 of 22 different watershed sites over a 19-month period. The incidence of *E. coli* O157 increased significantly when heavy rain caused an increased flow rate in the rivers.

In a study by Dingman (1999), cider samples were obtained from 11 cider mills operating in Connecticut during the 1997 to 1998 production season to test for the presence of *E. coli*. Three hundred fourteen cider samples were tested, out of which 11 (4%) were found to contain *E. coli*. Of the 11 mills, 6 (55%) tested positive for *E. coli* in the cider at least once during the production year. The microbiological quality of RTE food products sold in Taiwan was determined in a total of 164 samples of RTE food products, purchased in 1999–2000 from convenience stores and supermarkets in central Taiwan (Fang et al., 2003). The incidence of *E. coli* in these RTE food products was found to be 7.9%. Among the five types of food products tested, the highest incidence of *E. coli* (16%) was detected in hand-rolled sushi and food products made from ham.

More recently, a study was carried out by the Health Protection Agency and LACORS (2009) in 3,735 samples of RTE dried seeds, where 1.5% seed samples were found to contain unsatisfactory levels of *E. coli*, an indicator of poor hygiene. Retail samples of seeds of different varieties (alfalfa, hemp, linseed, melon, poppy, pumpkin, sesame, sunflower, other [watermelon, celery] and mixed seeds) were collected from supermarkets, health food shops, convenience shops, and market stalls. *Escherichia coli* was detected in melon, pumpkin, sesame, hemp, poppy, linseed, sunflower, and mixed seeds. The most frequently contaminated seed type was melon seeds.

1.4.3 *Strategies for controlling* E. coli *O157:H7 in RTE foods*

Although outbreaks from *E. coli* O157:H7 are often associated with ground beef products, other food products have also been implicated, and these include salami, sprouts, lettuce, unpasteurized apple juice, unpasteurized milk, yogurt, and cheese. Since most of these foods are considered to be RTE with no final cooking step, control of the disease from these products is difficult. Since the infectious dose of *E. coli* O157:H7 is very low (Tilden et al., 1996), any survival in an RTE product has the potential to cause illness. *Escherichia coli* O157:H7 may be present in raw ground beef that is used in the manufacturing of other RTE meat products (Alexander et al., 1994). Appropriate measures must therefore be taken to control *E. coli* O157:H7 in the final product. Following good farm and manufacturing practices could be the first step to reducing the risk of contamination with *E. coli* O157:H7. In

a recent FDA report, surface waterways exposed to cattle feces were identified as potential environmental risk factors for *E. coli* O157:H7 contamination of spinach associated with the 2006 outbreak (USFDA, 2007). Several reported outbreaks of *E. coli* O157:H7 in the United States between 1986 and 1996 associated with fruits and vegetables were also due to contamination of produce with animal manure, contamination of the water supply used to wash produce, or by cross-contamination of the fresh produce with raw meat products during meal preparation (Beuchat, 1996). The prevention of cross-contamination from raw beef to RTE products is therefore also critical. In addition, proper personal hygiene practices should be followed by workers at the pre- and post-harvest levels during handling of RTE products since even low doses can cause severe illness.

Several intervention strategies to reduce the prevalence of *E. coli* O157:H7 in meat are currently practiced by the food industry, including the HACCP program. According to the USDA regulation established in 1994, any raw ground beef and non-intact cuts of beef contaminated with *E. coli* O157:H7 are considered to be adulterated. To minimize contamination of food products that generally enter the food chain in the raw state, prevention of fecal contamination during slaughter, milking procedure, and pre- and post-harvest processing of fresh produce is important. Washing of hides prior to removal has been shown to significantly reduce levels of contamination in beef products (Ahmadia et al., 2006; Bosilevac et al., 2004; Sheridan, 2007). Decontamination strategies, which include the use of FDA- and USDA-approved antimicrobial treatments to reduce *E. coli* O157:H7 counts on carcass surfaces, hot water or steam treatments, and steam vacuuming, are some of the procedures that can eliminate or significantly reduce the numbers of the organism on meat surfaces.

Escherichia coli O157:H7 is also known to be acid tolerant and survives well in fermented and acidic foods. Studies have shown that *E. coli* O157:H7 can survive many of the typical dry fermentation processing conditions (Faith et al., 1998; Glass et al., 1992; Tilden et al., 1996). The organism's tolerance to acidic conditions has also been reported in the processing of other foods such as apple cider (Miller and Kaspar, 1994) and mayonnaise (Zhao and Doyle, 1994). These findings have led to significant changes in the industry and in the manufacturing of dry fermented sausage in the United States (Tilden et al., 1996). In 1996, the USDA-FSIS recommended that deli meat manufacturers adopt one of five options for the control of *E. coli* when they make fermented sausages (Blue Ribbon Task Force, 1996).

Pasteurization or other risk-reducing strategies, such as labeling, irradiation, disinfection, and sprays, for final product or ingredients are currently being considered by several countries. For fresh produce that is

consumed raw, good agricultural practices that significantly reduce the potential for fecal contamination of the crops growing in the fields is critical. In 2005 and 2006, produce-specific guidelines to minimize the potential for product contamination with *E. coli* O157:H7 were published by the FDA for fresh produce, including tomatoes, melons, lettuce, and leafy greens. For example, pasteurization of fruit juices was suggested as an effective control measure for these products, since *E. coli* O157:H7 is more sensitive to heat than is *Salmonella*. The range of temperatures that support growth of *E. coli* O157:H7 is more limited than that for generic *E. coli*, with a minimum of 46°F (8°C) and a maximum of 113°F (45°C). Interventions currently used by the fresh produce industry to reduce foodborne pathogens include several physical and chemical treatments (Gutierrez et al., 2008; Periago and Moezelaar, 2001). Fresh-cut lettuce and leafy greens go through one or more vigorous washing processes before they are packaged and sold to consumers. A wide range of sanitizers including ozone, peroxyacetic acid, chlorine dioxide, chlorinated trisodium phosphate, oxidized water, and acidified sodium chlorite have been evaluated for their ability to reduce *E. coli* O157:H7 on fresh produce (Stopforth et al., 2008). A number of studies have investigated efficacies of various antimicrobials on fresh produce such as tomatoes, lettuce, broccoli, and apples (Beuchat et al., 2001; Kilonzo-Nthenge et al., 2006) against *E. coli* O157:H7. However, these treatments do not always eliminate pathogenic microorganisms. For example, efforts by the industry to decrease contamination of sprouts have had limited success (Brooks et al., 2001; Taormina et al., 1999). Scientific studies have demonstrated that washing produce with water or a chlorine-based solution reduces *E. coli* O157 counts only modestly (Beuchat, 1999; Beuchat et al., 2004; Beuchat and Ryu, 1997). Water used in post-harvest operations to wash fresh produce may itself become a source of contamination if it contains pathogenic microorganisms and if there is insufficient wash water disinfectant present (Solomon et al., 2003). Therefore, once consumers obtain contaminated produce intended for raw consumption, little can be done to prevent illness. Until effective measures for preventing *E. coli* O157 contamination of produce items such as lettuce, cabbage, and sprouts can be implemented, consumers should be educated about potential risks associated with consuming these items raw. Further regulatory and educational efforts are needed to improve the safety of produce items.

Government regulatory agencies in the United States such as the USDA and the FDA have also taken steps to reduce the incidences of foodborne illnesses associated with bacterial pathogens, including *E. coli* O157:H7. Following the large multistate outbreak of *E. coli* O157 infections in 1993 in the western United States, a surveillance network called PulseNet was developed, in order to prevent future severe outbreaks.

PulseNet is the national network for molecular sub-typing of foodborne bacteria, coordinated by CDC. The participating laboratories include state health departments, some local health departments, USDA, and FDA. Another surveillance network established by the CDC is the Foodborne Diseases Active Surveillance Network (FoodNet), which is a joint effort by 10 state health departments, the USDA, and FDA. FoodNet conducts active surveillance for foodborne diseases and also conducts related epidemiologic studies to look at both sporadic cases and outbreaks to help better understand the epidemiology of foodborne illnesses and target effective prevention strategies.

1.5 Clostridium perfringens

Clostridium perfringens is an anaerobic (microaerophilic), Gram-positive, non-motile, spore-forming rod. The organism is widely distributed in the environment and is found in soil, dust, and raw ingredients such as spices, used in food processing, and in the intestinal tract of humans and animals (ICMSF, 1996; Juneja, Thippareddi, and Friedman, 2006). According to Smith and Williams (1984), it may be one of the most widely occurring bacterial pathogens. Raw protein foods of animal origin are frequently contaminated with *C. perfringens*. While the organism has the ability to produce enterotoxin (CPE), a large proportion of *C. perfringens* found in raw foods is CPE-negative (Saito, 1990). The CPE gene associated with food poisoning is commonly located on the chromosome while a non-food poisoning CPE gene is commonly located on a plasmid (Collie and McClane, 1998).

Clostridium perfringens food poisoning typically occurs from the ingestion of $>10^6$ viable vegetative cells of the organism in temperature-abused RTE meat products and is one of the most common types of foodborne illnesses (Labbe, 1989; Labbe and Juneja, 2002). Acidic conditions encountered in the stomach may actually trigger the initial stages of sporulation of the vegetative cells of *C. perfringens*. Once in the small intestine, the vegetative cells sporulate, releasing an enterotoxin upon sporangial autolysis. The enterotoxin is responsible for the pathological effects in humans as well as the typical symptoms of acute diarrhea with severe abdominal cramps and pain (Duncan and Strong, 1969; Duncan et al., 1972). Pyrexia and vomiting are usually not encountered in affected individuals. The typical incubation period before onset of symptoms is 8 to 24 hours, and acute symptoms usually last less than 24 hours. Full recovery within 24 to 48 hours is normal. Fatalities are rare in healthy individuals. Foodborne outbreaks of *C. perfringens* can be confirmed if $>10^5$ CFU/g of the organism or $>10^6$ spores/g are detected in the implicated food or feces, respectively (Labbe and Juneja, 2002).

Intrinsic and extrinsic factors that affect survival and growth of *C. perfringens* in food products and contribute to outbreaks include temperature, oxygen, water activity (a_w), pH, and curing salts. *Clostridium perfringens* strains that carry the CPE gene on the chromosome have previously been reported to be 60 times more heat resistant than the non-food poisoning strains (Sarker et al., 2000).

Heat treatment applied to raw product during preparation of RTE meat or poultry products is usually sufficient to kill vegetative cells of *C. perfringens* but not the spores present in the raw product. Likewise, slow cooking associated with low-temperature, long-time cooking will not eliminate *C. perfringens* spores. Cooking temperatures, if designed to inactivate *C. perfringens* spores, may negatively affect the product quality and desirable organoleptic attributes of foods. Therefore, spores are likely to survive the normal pasteurization/cooking temperatures applied to RTE foods. The surviving, heat-activated *C. perfringens* spores may germinate, outgrow, and multiply into large number of vegetative cells in cooked RTE foods if the rate and extent of cooling are not sufficient or if the processed foods are temperature abused. The abuse may occur during transportation, distribution, storage, or handling in supermarkets or during preparation of foods by consumers, which includes low-temperature, long-time cooking of foods as well as the scenarios when the foods are kept on warming trays before final heating or reheating. Thus, meat-containing RTE foods can be contaminated by vegetative cells that start out as spores originating in the raw meat or spices used in their production. In addition, cooking usually increases the anaerobic environment in food and reduces the number of competing spoilage organisms, which is ecologically important because *C. perfringens* competes poorly with the spoilage flora of many foods. Mean generation times in autoclaved ground beef during slow heating from 35°C to 52°C ranged from 13 to 30 minutes with temperature increases of 6°C to 12.5°C per hour (Willardsen et al., 1978). Another study also demonstrated growth of the organism in autoclaved ground beef during linear temperature increases (4.1°C–7.5°C per hour) from 25°C to 50°C (Roy et al., 1981).

Studies have described growth of *C. perfringens* during cooling of cooked RTE, uncured meat products. In a study by Tuomi et al. (1974), when cooked ground beef gravy inoculated with a mixture of vegetative cells and spores of *C. perfringens* NCTC 8239 was cooled in a refrigerator, rapid growth of the organism was reported to occur during the first 6 hours of cooling when the gravy temperature was in an ideal growth temperature range. Shigehisa et al. (1985) reported on germination and growth characteristics of *C. perfringens* spores inoculated into ground beef at 60°C and cooled to 15°C at a linear cooling rate of 5°C to 25°C per hour. They observed that the organism did not grow during exposure to falling

temperature rates of 25°C to 15°C per hour. However, multiplication of the organism was observed when the rate was less than 15°C per hour. Interestingly, the total population did not change for the first 150 minutes, regardless of the cooling rate. This study is not totally applicable to typical retail food operations because cooling is not linear; it is exponential. Steele and Wright (2001) evaluated growth of *C. perfringens* spores in turkey roasts cooked to an internal temperature of 72°C, followed by cooling in a walk-in cooler from 48.9°C to 12.8°C in 6, 8, or 10 hours. Results of that study indicated that an 8.9-hour cooling period was adequate to prevent growth of *C. perfringens* with a 95% tolerance interval. To simulate commercial chili cooling procedures, Blankenship et al. (1988) conducted exponential cooling experiments in which the cooling time was 4 hours and 6 hours for a temperature decline from 50°C to 25°C. This is approximately equivalent to a cooling rate of 12 hours and 18 hours for temperatures of 54.4°C to 7.2°C. They observed a declining growth rate in the case of 4 hours and 6 hours cooling time. Juneja et al. (1994) reported that no appreciable growth (<1.0 $\log_{10}$ CFU/g) occurred if cooling took 15 hours or less when cooked ground beef, inoculated with heat activated *C. perfringens* spores, was cooled from 54.4°C to 7.2°C at an exponential rate. However, *C. perfringens* grew by 4 to 5 $\log_{10}$ CFU/g if the cooling time was greater than 18 hours. This implies that *C. perfringens* is capable of rapid growth in meat systems, making this organism a particular concern to meat processors, as well as to the food service industry.

A limited amount of published research is available regarding growth of the pathogen in cooked cured meats during cooling. Taormina et al. (2003) inoculated bologna and ham batter with *C. perfringens* spores, followed by cooking and either cooling procedures typically used in industry or extended chilling. In that study, growth of the organism was not detected in any of the products tested during chilling from 54.4°C to 7.2°C. Zaika (2003) reported complete inhibition of *C. perfringens* germination and growth in cured ham with NaCl concentrations of 3.1% when cooled exponentially from 54.4°C to 7.2°C within 15, 18, or 21 hours. Cooked cured turkey cooled from 48.9°C to 12.8°C did not support *C. perfringens* growth in 6 hours; however, a 3.07-log increase was observed following 24 hours cooling time (Kalinowski et al., 2003).

1.5.1 *Outbreaks of* C. perfringens *in RTE foods*

The incidence of *Clostridium perfringens* gastrointestinal illnesses in the United States has been estimated by the CDC to be 248,000 cases per year leading to 41 hospitalizations and 7 deaths each year, with 100% of these cases being due to foodborne transmission of the pathogen (Mead et al., 1999) and with an estimated cost of $200 per case (Todd, 1989). It is currently

estimated that the incidence of foodborne illness from *C. perfringens* is a factor of 10 to 350 times the number of cases actually reported (Mead et al., 1999). In another report from 1993 to 1997, *C. perfringens* accounted for 2.1% of the outbreaks and 3.2% of the cases of foodborne illnesses and was the third most common cause of confirmed outbreaks and cases of foodborne illness (Olsen, 2000). There is no evidence to suggest that the contrary exists today. Most cases of *C. perfringens* food poisoning are mild and are not reported. In 1994, the total cost of illnesses due to *C. perfringens* was estimated at $123 million in the United States (Anonymous, 1995). The estimated large number of illnesses due to *C. perfringens* clearly stresses the importance of cooling foods quickly after cooking with proper refrigeration during shelf-life storage.

Clostridium perfringens outbreaks usually result from improper handling and preparation of foods, such as inadequate cooling, at the home, retail, or food service level, rarely involving commercial meat processors (Bean et al., 1997; Bean and Griffin, 1990; Bryan, 1988; CDC, 2000; Taormina et al., 2003). Major contributing factors leading to food poisoning associated with *C. perfringens* include its ability to form heat-resistant spores that can survive commercial cooking operations, as well as the ability to germinate, outgrow, and multiply at a very rapid rate during post-cook handling, primarily under conditions conducive to germination. Such conditions occur when the cooling of large batches of cooked foods is not fast enough to inhibit bacterial growth or the foods are held at room temperature for an extended period or are temperature abused. Germination and outgrowth of *C. perfringens* spores during cooling of thermally processed meat products has been reported extensively (Juneja et al., 1994, 1999). Accordingly, improper cooling (40.9%) of food products has been cited as the most common cause of *C. perfringens* outbreaks (Angulo et al., 1998).

Meat and poultry products were associated with the vast majority of *C. perfringens* outbreaks in the United States, probably due to the fastidious requirement for more than a dozen amino acids and several vitamins for the organism to grow in these products (Brynestad and Granum, 2002; Labbe and Juneja, 2002). Roast beef, turkey, meat-containing Mexican foods, and other meat dishes have been associated with *C. perfringens* food-poisoning outbreaks (Bryan, 1969, 1988). Roast beef and other types of cooked beef were primarily implicated as vehicles of transmission for 26.8% of 190 *C. perfringens* enteritis outbreaks in the United States from 1973 to 1987 and 33.9% of 115 outbreaks from 1977 to 1984, although poultry products were also commonly implicated (Bean and Griffin, 1990; Bryan, 1988). In retrospect, most outbreaks of *C. perfringens* food poisoning can be avoided by adequate cooking of meat products followed by holding at hot temperatures or rapid cooling.

1.5.2 *Incidence and prevalence of* C. perfringens *in RTE foods*

Clostridium perfringens is commonly found on vegetable products and in other raw and processed foods. The organism is frequently found in meats, generally through fecal contamination of carcasses, contamination from other ingredients such as spices, or post-processing contamination. *Clostridium perfringens* was detected in 36%, 80%, and 2% of fecal samples from cattle, poultry, and pigs, respectively (Tschirdewahn et al., 1991). The organism was found on 29%, 66%, and 35% of beef, pork, and lamb carcasses, respectively (Smart et al., 1979). *Clostridium perfringens* was isolated from 43.1% of processed and unprocessed meat samples tested in one study, including beef, veal, lamb, pork, and chicken products (Hall and Angelotti, 1965). Many areas within broiler chicken processing plants are contaminated with the organism (Craven, 2001), and the incidence of *C. perfringens* on raw poultry ranges from 10% to 80% (Waldroup, 1996). *Clostridium perfringens* was detected in 47.4% of raw ground beef samples (Ladiges et al., 1974), and a mean level of 45.1 *C. perfringens* per cm^2 was detected on raw beef carcass surface samples (Sheridan et al., 1996). *Clostridium perfringens* was detected on 38.9% of commercial pork sausage samples (Bauer et al., 1981), and on raw beef, equipment, and cooked beef in food service establishments (Bryan and McKinley, 1979). In a survey conducted in the United Kingdom by Hobbs et al. (1965), 67% of the vacuum-packaged fish products samples were positive for clostridia, predominantly *C. perfringens.* About 50% of raw or frozen meat and poultry contains *C. perfringens* (Labbe, 1989). *Clostridium perfringens* spores were isolated from 80% of 54 different spices and herbs (Deboer et al., 1985). This presents a public health hazard since spices and herbs are commonly used in meat and meat products. However, early surveys of foods did not determine the enterotoxin-producing ability of isolates. In recent surveys, on the incidence of *C. perfringens* in raw and processed foods, an incidence level of 30% to 80% has been found (Lin and Labbe, 2003).

To determine the level of contamination, Kalinowski et al. (2003) reported that out of 197 raw comminuted meat samples analyzed, all but two samples had undetectable levels (<3 spores per g) and two ground pork samples contained 3.3 and 66 spores per gram. In another survey, Taormina et al. (2003) examined a total of 445 whole muscle and ground or emulsified raw pork, beef, and chicken product mixtures acquired from industry sources for *C. perfringens* vegetative cells and spores. Out of 194 cured whole-muscle samples examined, 1.6% were positive for vegetative cells and spores were not detected. Out of 152 cured ground or emulsified samples, 48.7% and 5.3% were positive for vegetative cells and spores, respectively. Populations of vegetative cells and spores did not exceed 2.72 and 2.00 $\log_{10}$ CFU/g. These studies suggest a low incidence of spores in

raw, cured, whole-muscle ham as well as low levels of spores in cured, ground, emulsified meats, and in raw comminuted meat samples.

Li et al. (2007) surveyed soils and home kitchen surfaces in the Pittsburgh, Pennsylvania, area to determine the prevalence of *cpe*-positive *C. perfringens* isolates in these two environments and reported that neither soil nor home kitchen surfaces represent major reservoirs for type A isolates with chromosomal *cpe* that cause food poisoning, although soil does appear to be a reservoir for *cpe*-positive isolates causing non-foodborne gastrointestinal diseases. Rahmati and Labbe (2008) reported 17 samples positive for *C. perfringens*, one possessed enterotoxin gene, out of 347 fresh and processed seafood samples examined. In another study (Wen and McClane, 2004), a survey of American retail foods reported that approximately 1.7% of raw meat, fish, and poultry items sold in retail foods stores contained type A isolates carrying a chromosomal *cpe* gene, and no plasmid *cpe* gene was found in any of those surveyed retail foods. In a national survey of the retail meats conducted in Australia, *C. perfringens* was not recovered from any of the 94 ground beef samples and was isolated from 1 of 92 samples of diced lamb (Phillips et al., 2008). These surveys indicate a low incidence of *C. perfringens* in retail meats.

1.5.3 *Strategies for controlling* C. perfringens *in RTE foods*

Due to its ubiquitous nature and rapid growth in meat products, *C. perfringens* can be a potential hazard in processed meat and poultry products. The FDA (2001b) Food Code dictates that cooked, potentially hazardous foods such as meats should be cooled from 60°C to 21°C within 2 hours and from 60°C to 5°C within 6 hours. In the United Kingdom, it is recommended that uncured cooked meats be cooled from 50°C to 12°C within 6 hours and from 12°C to 5°C within 1 hour (Gaze et al., 1998). Safe cooling times for cured meats may be up to 25% longer (Gaze et al., 1998). The USDA-FSIS compliance guidelines (USDA-FSIS, 2001) for chilling of thermally processed meat and poultry products state that these products should be chilled following the prescribed chilling rates, or require that process authorities validate the safety of customized chilling rates to control spore-forming bacteria. Specifically, the guidelines state that cooling from 54.4°C to 26.7°C should take no longer than 1.5 hours, and cooling from 26.7°C to 4.4°C should take no longer than 5 hours (USDA-FSIS 2001). Additional guidelines allow for the cooling of certain cured cooked meats from 54.4°C to 26.7°C in 5 hours and from 26.7°C to 7.2°C in 10 hours (USDA-FSIS 2001). If meat processors are unable to meet the prescribed time-temperature cooling schedule, they must be able to document that the customized or alternative cooling regimen used will result in a less than 1-$\log_{10}$ CFU increase in *C. perfringens* in the finished product. If the cooling

guidelines cannot be achieved, computer modeling, product sampling, or both, can be used to evaluate the severity and microbiological risk of the process deviation, and additional challenge studies may be necessary to determine if performance standards have been met.

The presence of inhibitory agents in the products can affect germination of *C. perfringens* spores and may also affect the minimum growth temperatures for the germinated spores. Recent studies have shown the efficacy of certain antimicrobial agents against the growth of *C. perfringens* during cooling of meat products. For instance, Sabah, Thippareddi, et al. (2003) found that 0.5% to 4.8% sodium citrate inhibited growth of *C. perfringens* in cooked, vacuum-packaged, restructured beef cooled from 54.4°C to 7.2°C within 18 hours. The same researchers also demonstrated growth inhibition of the microorganism by oregano in combination with organic acids during cooling of *sous-vide* cooked ground beef products (Sabah, Juneja, et al., 2003). Organic acid salts such as 1% to 1.5% sodium lactate, 1% sodium acetate, 0.75% to 1.3% buffered sodium citrate (with or without sodium diacetate), 1.5% sodium lactate supplemented with sodium diacetate inhibited germination and outgrowth of *C. perfringens* spores during the chilling of marinated ground turkey breast (Juneja and Thippareddi, 2004a, 2004b). In another study, Thippareddi et al. (2003) reported complete inhibition of *C. perfringens* spore germination and outgrowth by sodium salts of lactic and citric acids (2.5% and 1.3%, respectively) in roast beef, pork ham, and injected turkey products. Incorporation of plant-derived natural antimicrobials, such as thymol (1%–2%), cinnamaldehyde (0.5%–2%), oregano oil (2%), and carvacrol (1%–2%), as well as the biopolymer chitosan (0.5%–3%) derived from shellfish, and green tea catechins (0.5%–3%) individually inhibited *C. perfringens* germination and outgrowth during exponential cooling of ground beef and turkey (Juneja, Thippareddi, Bari, et al., 2006; Juneja, Thippareddi, and Friedman, 2006; Juneja and Friedman, 2007; Juneja et al., 2007). Therefore, natural compounds can be used as ingredients in processed meat products to provide an additional measure of safety to address the *C. perfringens* hazard during chilling and subsequent refrigeration of meat products, thus further minimizing risk to the consumer.

Numerous studies have examined the heat resistance of *C. perfringens* spores, vegetative cells, or both. Heat resistance varies among strains of *C. perfringens*, although both heat-resistant and heat-sensitive strains can cause food poisoning (Labbe and Juneja, 2006). Environmental stresses, such as storage and holding temperatures, and low-temperature, long-time cooking expose the contaminating vegetative and spore-forming foodborne pathogens to conditions similar to heat shock, thereby rendering the heat-shocked organisms more resistant to subsequent lethal heat treatments. Researchers have reported on the quantitative assessment of

heat resistance, the heat shock response, and the induced thermotolerance to assist food processors in designing thermal processes for the inactivation of *C. perfringens*, thereby ensuring the microbiological safety of cooked foods (Juneja, Novak, Eblen, et al., 2001; Juneja et al., 2003). Heat shocking vegetative cells suspended in beef gravy at 48°C for 10 minutes allowed the microorganisms to survive longer and increased the heat resistance to as high as 1.5-fold (Juneja, Novak, Eblen, et al., 2001). The thermal resistance (D-values in minutes) of *C. perfringens* cells heated in beef gravy at 58°C ranged from 1.21 minutes (C 1841 isolate) to 1.60 minutes (F 4969 isolate). Compared to the control (no heat shock), the increase in heat resistance after heat shocking at 58°C ranged from 1.2-fold (B 40 isolate) to 1.5-fold (NCTC 8239 isolate). The D-values of *C. perfringens* spores heated in beef gravy at 100°C ranged from 15.50 minutes (NCTC 8239 isolate) to 21.40 minutes (NB 16 isolate) (Juneja et al., 2003). Compared to the control (no heat shock), the increase in heat resistance of *C. perfringens* spores at 100°C after heat shocking ranged from 1.1-fold (NCTC 8238 isolate) to 1.5-fold (F 4969 isolate). Similar results were obtained by Heredia et al. (1997), who found that a sublethal heat shock at 55°C for 30 minutes increased tolerance of both spores and vegetative cells to a subsequent heat treatment. In their study, when the heat resistance of *C. perfringens* vegetative cells, grown at 43°C in fluid thioglycollate broth (FTG) to an A_{600} of 0.4 to 0.6, was determined, the D-values at 55°C of 9 and 5 minutes for FD-1 and FD1041 strains, respectively, were reported. The D-values of the heat-shocked cells were 85 and 10 minutes, respectively. Heredia et al. (1997) heat shocked sporulating cells of *C. perfringens* at 50°C for 30 minutes and then determined the D-values at 85°C or 90°C. The authors reported that a sublethal heat shock increased the thermotolerance of *C. perfringens* spores by at least 1.7- to 1.9-fold; the D-values at 85°C increased from 24 to 46 minutes, and at 95°C, from 46 to 92 minutes, respectively. Bradshaw et al. (1982) reported D-values at 99°C for *C. perfringens* spores suspended in commercial beef gravy ranging from 26 to 31.4 minutes. Miwa et al. (2002) found that spores of enterotoxin-positive *C. perfringens* strains were more heat-resistant than were enterotoxin-negative strains. Similarly, food poisoning isolates of the organism are generally more heat-resistant than *C. perfringens* spores from other sources (Labbe, 1989). Sarker et al. (2000) reported D-values at 100°C for 12 isolates of *C. perfringens* spores, carrying either chromosomal *cpe* gene or plasmid *cpe* gene, in DS medium ranged from 0.5 minutes to 124 minutes. Sarker et al. (2000) reported D-values at 55°C for *C. perfringens* cells grown at 37°C in FTG of 12.1 minutes and 5.6 minutes for E13 and F5603 strains, respectively. These authors also reported a strong association of the food poisoning isolates and increased heat resistance; the D-values at 55°C or 57°C for the *C. perfringens* chromosomal *cpe* isolates were significantly higher

($p < 0.05$) than the D-values of the *C. perfringens* isolates carrying a plasmid *cpe* gene; however, differences in heat resistance were not observed at higher temperatures. Nevertheless, understanding these variations in heat resistance is certainly necessary in order to design adequate cooking regimes to eliminate *C. perfringens* vegetative cells in RTE foods.

Studies have been conducted to assess the effects and interactions of multiple food formulation factors on the heat resistance of spores and vegetative cells of *C. perfringens*. In a study by Juneja and Marmer (1996), when the thermal resistance of *C. perfringens* spores (expressed as D-values in minutes) in turkey slurries that included 0.3% sodium pyrophosphate at pH 6.0 and salt levels of 0%, 1%, 2%, or 3% was assessed, the D-values at 99°C decreased from 23.2 minutes (no salt) to 17.7 minutes (3% salt). In a beef slurry, the D-values significantly decreased ($p < 0.05$) from 23.3 minutes (pH 7.0, 3% salt) to 14.0 minutes (pH 5.5, 3% salt) at 99°C (Juneja and Majka, 1995). While addition of increasing levels (1%–3%) of salt in turkey (Juneja and Marmer, 1996) or a combination of 3% salt and pH 5.5 in beef (Juneja and Majka, 1995) can result in a parallel increase in sensitivity of *C. perfringens* spores at 99°C, mild heat treatments given to minimally processed foods will not eliminate *C. perfringens* spores. Juneja and Marmer (1998) examined the heat resistance of vegetative *C. perfringens* cells in ground beef and turkey containing sodium pyrophosphate (SPP). The D-values in beef that included no SPP were 21.6, 10.2, 5.3, and 1.6 minutes at 55°C, 57.5°C, 60°C, and 62.5°C, respectively; the values in turkey ranged from 17.5 minutes at 55°C to 1.3 minutes at 62.5°C. Addition of 0.15% SPP resulted in a concomitant decrease in heat resistance as evidenced by reduced bacterial D-values. The D-values in beef that included 0.15% SPP were 17.9, 9.4, 3.5, and 1.2 minutes at 55°C, 57.5°C, 60°C, and 62.5°C, respectively; the values in turkey ranged from 16.2 minutes at 55°C to 1.1 minutes at 62.5°C. The heat resistance was further decreased when the SPP level in beef and turkey was increased to 0.3%. Heating such products to an internal temperature of 65°C for 1 minute killed >8 $\log_{10}$ CFU/g. The z-values in beef and turkey for all treatments were similar, ranging from 6.22°C to 6.77°C. Thermal death time values from this study should assist institutional food service settings in designing thermal processes that ensure safety against *C. perfringens* in cooked beef and turkey.

Researchers have assessed the efficacy of added preservatives on inhibiting or delaying the growth of *C. perfringens* in extended shelf-life, refrigerated, processed meat products. When ground turkey containing 0.3% sodium pyrophosphate and 0%, 1%, 2%, or 3% salt was *sous-vide* processed (71.1°C) and held at 28°C, lag times of 7.3, 10.6, 11.6, and 8.0 hours were observed for salt level of 0%, 1%, 2%, and 3%, respectively (Juneja and Marmer 1996). Growth of *C. perfringens* spores in cooked ground turkey with added 0.3% sodium pyrophosphate was inhibited for 12 hours at 3%

salt, pH 6.0, and 28°C. After 16 hours, spores germinated and grew at 28°C from 2.25 to >5 $\log_{10}$ CFU/g in *sous-vide* processed (71.1°C) turkey samples regardless of the presence or absence of salt (Juneja and Marmer, 1996). While *C. perfringens* spores germinated and grew at 15°C to >5 $\log_{10}$ CFU/g in turkey with no salt by day 4, the presence of 3% salt in samples at 15°C completely inhibited germination and subsequent multiplication of vegetative cells even after 7 days of storage (Juneja and Marmer 1996). Thus, the addition of 3% salt in *sous-vide* processed ground turkey containing 0.3% SPP delayed growth for 12 hours at 28°C and completely inhibited the outgrowth of spores at 15°C (Juneja and Marmer 1996). However, 3% salt in RTE products will not inhibit germination and growth of *C. perfringens* spores if refrigerated products are temperature abused to 28°C for an extended period. In another study (Juneja and Majka, 1995) the combination of 3% salt and pH 5.5 inhibited *C. perfringens* growth from spores in *sous-vide* processed ground beef supplemented with 0.3% SPP at 15°C and 28°C. Growth from *C. perfringens* spores occurred within 6 days in *sous-vide* processed (71.1°C) pH 7.0 ground beef samples, but was delayed until day 8 in the presence of 3% salt at pH 5.5 at 15°C (Juneja and Majka, 1995). *C. perfringens* growth from a spore inoculum at 4°C was not observed in *sous-vide* cooked turkey or beef samples (Juneja and Majka, 1995; Juneja and Marmer, 1996). In a related study, Juneja et al. (1996) showed that *C. perfringens* growth in cooked turkey can be effectively inhibited in an atmosphere containing 25% to 75% CO_2, 20% O_2, and balance N_2 in conjunction with good refrigeration; however, the atmosphere cannot be relied upon to eliminate the risk of *C. perfringens* food poisoning in the absence of proper refrigeration (Juneja et al., 1996). Kalinowski et al. (2003) investigated the fate of *C. perfringens* in cooked-cured and uncured turkey at refrigeration temperatures. In their study, *C. perfringens* decreased by 2.52, 2.54, and 2.75 $\log_{10}$ CFU/g in cured turkey held at 0.6°C, 4.4°C, and 10°C, respectively, and the reductions in levels were similar in uncured turkey.

The efficacy of sodium lactate (NaL) in inhibiting the growth from spores of *C. perfringens* in a *sous-vide* processed food has been assessed. Inclusion of 3% NaL in *sous-vide* beef goulash inhibited *C. perfringens* growth at 15°C, delayed growth for a week at 20°C, and had little inhibitory effect at 25°C (Aran 2001). While addition of 4.8% NaL restricted *C. perfringens* growth from spores for 480 hours at 25°C in *sous-vide* processed (71.1°C) marinated chicken breast, it delayed growth for 648 hours at 19°C. *Clostridium perfringens* growth was not observed at 4°C regardless of NaL concentration (Juneja, 2006). These studies suggest that NaL can have significant bacteriostatic activity against *C. perfringens* and may provide *sous-vide* processed foods with a degree of protection against this microorganism, particularly if employed in conjunction with adequate refrigeration.

Predictive bacterial growth models that describe *C. perfringens* spore germination and outgrowth during cooling of food systems have been generated by researchers using constant temperature data. Juneja et al. (1999) presented a model for predicting the relative growth of *C. perfringens* from spores, through lag, exponential, and stationary phases of growth, at temperatures spanning the entire growth temperature range of about 10°C to 50°C. Huang (2003a, 2003b, 2003c) used different mathematical methods to estimate the growth kinetics of *C. perfringens* in ground beef during isothermal, square-waved, linear, exponential, and fluctuating cooling temperature profiles. Juneja, Novak, Marks, et al. (2001) developed a predictive cooling model for cooked, cured beef based on growth rates of the organism at different temperatures. This model estimated that exponential cooling from 51°C to 11°C in 6, 8, or 10 hours would result in an increase of 1.43, 3.17, and 11.8 $\log_{10}$ CFU/g, respectively, assuming that the ratio of the mathematical lag time to the generation time for cells in exponential phase of growth was equal to 8.068, the estimated geometric mean. A similar model was later developed for cooked, cured chicken (Juneja and Marks, 2002). Juneja, Huang, et al. (2006) also developed a model for predicting growth of *C. perfringens* from spore inocula in cured pork ham. In their study, isothermal growth of *C. perfringens* at various temperatures from 10°C to 48.9°C were evaluated using a methodology that employed a numerical technique to solve a set of differential equations, simulating the dynamics of bacterial growth; the authors described the effect of temperature on the kinetic parameter k_D by the modified Ratkowski model. According to the coefficient of the model, the estimated theoretical minimum and maximum growth temperatures of *C. perfringens* in cooked cured pork were 13.5°C and 50.6°C, respectively. The kinetic and growth parameters obtained from these studies can be used in evaluating growth of *C. perfringens* from spore populations during dynamically changing temperature conditions such as those encountered in meat processing.

In a model for growth of *C. perfringens* during cooling of cooked uncured beef (Juneja et al., 2008), for a temperature decline from 54.4°C to 27°C in 1.5 hours, a $\log_{10}$ relative growth of about 1.1 was predicted, with a standard error of about 0.08 $\log_{10}$. While the observed results for two replicates were 0.43 and 0.90 $\log_{10}$, for the same temperature decline in 3 hours, the predicted $\log_{10}$ relative growth was about 3.6 $\log_{10}$ (with a standard error of about 0.07), and the observed $\log_{10}$ relative growths were 2.4 $\log_{10}$ and 2.5 $\log_{10}$. When the cooling scenarios extended to lower temperature, the predictions were somewhat better, taking into account the larger relative growth: for a cooling scenario of 54.4°C to 27°C in 1.5 hours and 27°C to 4°C in 12.5 hours, the average observed and predicted

$\log_{10}$ relative growths were 2.7 $\log_{10}$ and 3.2 $\log_{10}$, respectively; when cooling was extended from 27°C to 4°C in 15 hours, the average observed and predicted $\log_{10}$ relative growths were 3.6 $\log_{10}$ and 3.7 $\log_{10}$, respectively. For the latter cooling scenario the levels were greater than 6 $\log_{10}$, still less than stationary levels of about 7 $\log_{10}$ or 8 $\log_{10}$. The differences of the estimates obtained for the models were insignificant. Smith-Simpson and Schaffner (2005) collected data under changing temperature conditions and developed a model to predict growth of *C. perfringens* in cooked beef during cooling. It was suggested that the accuracy of the germination, outgrowth, and lag (GOL) time model has a profound influence upon the overall prediction, with small differences in GOL time prediction (~1 hour) having a very large effect on the predicted final concentration of *C. perfringens*. Amezquita et al. (2004) developed an integrated model for heat transfer and dynamic growth of *C. perfringens* during cooling of cured ham and demonstrated that the effective integration of engineering and microbial modeling is a useful quantitative tool for ensuring microbiological safety. The previously mentioned models can be successfully used to design microbiologically "safe" cooling regimes for cooked meat and poultry products.

Recent research has focused on combining traditional inactivation, survival, and growth-limiting factors at sub-inhibitory levels with emerging novel non-thermal intervention food preservation techniques using ionizing radiation, high hydrostatic pressure, or exposure to ozone. For example, the efficacy of high pressure is considerably enhanced when combined with heat, antimicrobials, or ionizing radiation. The effect of the combined intervention strategies is either additive or synergistic in which the interaction leads to a combined effect of greater magnitude than the sum of the constraints applied individually. For example, the lethal effect of heat on spores can be enhanced after exposure to ozone. Novak and Yuan (2004) reported that the spores were more sensitive to heat at 55°C or 75°C following 5 ppm of aqueous ozone for 5 minutes. Shelf-life extension of meat processed with 5 ppm O_3 for 5 minutes and containing *C. perfringens* spores combined with modified atmosphere packaging as a "hurdle" technology was proven to be effective in inhibiting spore germination and outgrowth over 10 days storage at CO_2 concentrations above 30% and 4°C (Novak and Yuan, 2004). Likewise, *C. perfringens* cells exposed to 3 ppm O_3 for 5 minutes following mild heat exposure (55°C for 30 min) were more susceptible to ozone treatment.

When *C. perfringens* spores were suspended in peptone solution and exposed to combination treatments of hydrostatic pressure (138–483 MPa), time (5 min), temperature (25°C–50°C), inactivation of spores ranged between 0.1 and 0.2 log cycles (Kalchayanand et al., 2004). When suspended

spores were pressurized at 50°C for 5 minutes and stored at 4°C or 25°C for 24 hours, 12% to 52% spores germinated, indicating that germination increased both at 4°C and 25°C during 24 hours. Log_{10} reductions of spores were 6.1 log/ml when bacteriocins were supplemented in the recovery medium. These results show that germinated spores at high levels could be killed by using a bacteriocin-based preservative in foods.

1.6 Conclusion

Ready-to-eat foods are becoming increasingly popular among the present-day consumers due to their convenience value. Since these foods are either minimally processed or are consumed raw, the microbiological risks associated with these products has also increased. The four major pathogens that have been repeatedly implicated in foodborne illnesses associated with these foods are *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7, and *C. perfringens*.

Among the four pathogens discussed in this chapter, *L. monocytogenes* has been historically the most common foodborne pathogen linked to RTE foods due to its hardy and ubiquitous nature and the difficulty in eradicating it from the food-processing environment. The high prevalence of *L. monocytogenes* in RTE products is a major threat to public health. Outbreaks associated with *L. monocytogenes* have prompted regulatory agencies to impose stringent regulations, with a USDA "zero tolerance" policy, in place since 1989, for *L. monocytogenes* in RTE meat products. The FSIS-USDA has also mandated three alternative approaches for controlling *L. monocytogenes* in RTE meats.

Salmonella and *E. coli* O157:H7 infections due to contamination of RTE foods are becoming increasingly common, especially in fresh produce. Since these microorganisms are part of the natural intestinal microflora of food animals, contamination of RTE food products from these organisms is easy. Additionally, both *Salmonella* and *E. coli* O157:H7 can survive extreme temperature, moisture, and pH conditions, with *Salmonella* being slightly more temperature and desiccation tolerant than *E. coli* O157:H7. These traits make these pathogens adept at surviving and proliferating in food-processing environments, making their control or elimination in RTE foods challenging.

Meat and poultry are the most common foods associated with *C. perfringens* food poisoning. Foodborne illness by this organism is usually a result of inadequate refrigeration and inadequate reheating of RTE meat and poultry products. Ingestion of large number of organisms in a contaminated food is necessary for the pathogen to survive passage through the stomach and for it to sporulate and elaborate enterotoxin in

the intestines. The preferred method for controlling the growth of this pathogen is not necessarily initial heating, but rather adequate cooling and adequate reheating following cooling to inactivate any cells produced due to bacterial multiplication during cooling. Control measures are in place that take advantage of the microorganism's limitations to growth with respect to oxygen, water activity, pH, curing salts, organic acids, and natural inhibitors. Recently, many predictive growth models have been developed to accurately estimate *C. perfringens* survival following various types of food-processing scenarios. The best strategy for controlling *C. perfringens* appears to be a hurdle approach combined with careful handling of foods to avoid temperature abuse. Regulatory requirements for *C. perfringens* in foods in the United States follow the USDA-FSIS compliance guidelines.

However, foodborne infections from these pathogens can be reduced by following a comprehensive farm-to-table approach to food safety. The critical links in this food safety chain are the farmers, industry, food inspectors, retailers, food service workers, and consumers, who can play a significant role in improving the microbiological safety of RTE foods.

1.7 Future outlook

The popularity of RTE foods and the resulting increased consumption calls for enhanced and food-specific measures to control or eliminate pathogens in these foods. Mitigation strategies are needed to reduce or prevent contamination during growing, harvesting, and processing of raw ingredients used in preparation of RTE foods. In addition, there is a critical need for continued education of food industry personnel at pre- and post-harvest processing levels and at the retail level, and that of consumers about risks and prevention measures. In particular, continued efforts are needed to understand factors contributing to contamination of fresh produce and processed foods. Evaluation of the safety of processing methods can ensure more reliable and consequently safer processes. Efforts also need to be directed toward development and implementation of effective intervention strategies. Control measures must be applied along the entire production chain, from farm, via processing, to the table, and applied as early as possible in the production chain. The epidemiology of several of the existing and potential foodborne pathogens is also poorly understood. New DNA-based tools for the specific detection/enumeration and identification of pathogenic bacteria can be useful in clarifying which species and sub-groups are of real concern.

Microorganisms can successfully adapt to changes in food production, processing, and preservation techniques, which can result in new and emerging foodborne pathogens and the re-emergence of pathogens with a

known history of causing foodborne illnesses. It is therefore important to meet the challenges, resulting from the extraordinary capability of foodborne pathogens to adapt to stressful conditions, through science-based research. However, not all of the challenges of preventing foodborne illness can be met by scientific research alone. New food safety policies in response to scientific research, which increase understanding of food safety hazards and a constantly changing food-processing industry, must also be put in place to reduce future foodborne diseases. Regulatory agencies such as the FDA need to be provided with adequate funding to conduct sufficient inspections, and the authority to mandate recalls. The laws, regulations, agencies, and organizations that are part of the food safety system are frequently not up to date with the current scientific knowledge of the risks associated with foodborne pathogens. It is therefore crucial to better understand these risk factors along the farm-to-fork continuum, along with the associated costs and benefits of implementing mitigation strategies that would help implement future systemic changes to enhance food safety.

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